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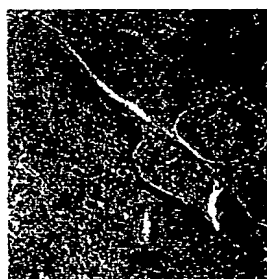
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- (71) Applicants and  
(72) Inventors: **BELSHAM, Denise** [CA/CA]; 190 Clendenan Avenue, Toronto, Ontario M6P 2X2 (CA). **LOVEJOY, David** [CA/CA]; 149 Baker Street, Stouffville, Ontario L4A 1K6 (CA).
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(54) Title: IMMORTALIZED HYPOTHALAMIC NEURONAL CELL LINES



(57) Abstract: The present invention is directed to a method of preparing cell lines of hypothalamic origin. The method involves infecting fetal hypothalamic cells with a retroviral vector harbouring a viral oncogene, preferably SV-40 large T antigen, followed by selection and cloning. A plurality of cell lines have been prepared which express a variety of neuronal markers. The cell lines of the present invention are useful in the development of experimental models and in the treatment of disease.

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 03/00621

A. CLASSIFICATION OF SUBJECT MATTER  
 IPC 7 C12N5/06 C07K14/575

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, EP0-Internal, MEDLINE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MELLON PAMELA L ET AL: "An immortal cell culture model of hypothalamic gonadotropin-releasing hormone neurons." METHODS (ORLANDO), vol. 7, no. 3, 1995, pages 303-310, XP002256788 ISSN: 1046-2023	1,2, 14-17
Y	abstract  page 305, right-hand column, line 12 - line 31 page 307, right-hand column, line 6 - line 18 page 309, left-hand column, line 21 - right-hand column, line 2 --- -/--	3-13, 18-21

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

## \* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 03/00621

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MELLON P L ET AL: "IMMORTALIZATION OF HYPOTHALAMIC GNRH NEURONS BY GENETICALLY TARGETED TUMORIGENESIS" NEURON, CAMBRIDGE, MA, US, vol. 5, no. 1, 1 July 1990 (1990-07-01), pages 1-10, XP009000953	1,2, 14-17
Y	abstract  page 1, right-hand column, line 16 - line 30 page 2, right-hand column, paragraph 2 figure 5 page 4, left-hand column page 5, right-hand column, line 1 -page 6, left-hand column, line 3 page 6, right-hand column, line 13 - line 17 page 8, left-hand column, line 29 -right-hand column, line 10	3-13, 18-21
X	--- QUINONES-JENAB V ET AL: "Cell cycle-specific expression of the neuronal phenotype in an immortal hypothalamic cell line." SOCIETY FOR NEUROSCIENCE ABSTRACTS, vol. 18, no. 1-2, 1992, page 768 XP009017695 22nd Annual Meeting of the Society for Neuroscience;Anaheim, California, USA; October 25-30, 1992 ISSN: 0190-5295	1,2, 14-17
Y	abstract	3-13, 18-21
X	--- TELLAM D J ET AL: "Direct regulation of GnRH transcription by CRF-like peptides in an immortalized neuronal cell line." NEUROREPORT. ENGLAND 5 OCT 1998, vol. 9, no. 14, 5 October 1998 (1998-10-05), pages 3135-3140, XP009017726 ISSN: 0959-4965	1,2, 14-17
Y	abstract  page 3136, left-hand column, line 10 - line 14 page 3139, right-hand column, line 19 - line 21 page 3140, left-hand column, paragraph 2 -right-hand column, paragraph 1	3-13, 18-21
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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 03/00621

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MELLON P L ET AL: "Immortalization of neuroendocrine cells by targeted oncogenesis." RECENT PROGRESS IN HORMONE RESEARCH. UNITED STATES 1991, vol. 47, 1991, pages 69-93;discussion 93 - 96, XP009017629 ISSN: 0079-9963	1,2, 14-17
Y	page 70, paragraph 4  page 71 page 78 -page 83 page 88, paragraph 5 page 89, paragraph 3 page 90, paragraph 2 - paragraph 3 ---	3-13, 18-21
A	WALDBIESER G C ET AL: "TISSUE-SPECIFIC EXPRESSION OF THE HUMAN NEUROPEPTIDE Y GENE IN TRANSGENIC MICE" MOLECULAR BRAIN RESEARCH, vol. 14, no. 1-2, 1992, pages 87-93, XP009018379 ISSN: 0169-328X the whole document ---	1-21
A	HIGUCHI H ET AL: "AGE-DEPENDENT INCREASE IN NEUROPEPTIDE Y GENE EXPRESSION IN RAT ADRENAL GLAND AND SPECIFIC BRAIN AREAS" JOURNAL OF NEUROCHEMISTRY, vol. 57, no. 6, 1991, pages 1840-1847, XP009018377 ISSN: 0022-3042 the whole document -----	1-21

# INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/CA 03/00621

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-17, 21 (partially)

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

This international Searching Authority found multiple (groups of) inventions in this international application as follows :

Invention 1 : claims 1-17,21 (partially); as far as applicable

Immortalized cell line of murin hypothalamic neuronal cells comprising a gene encoding polyoma virus large T antigen operably linked to a promoter and expressing the marker neuropeptide Y (NPY), methods for obtaining a neuropeptide or for identifying a modulator of a neuropeptide using said cell line.

Inventions 2-33 : claims 1-17 and 21; as far as applicable.

As invention 1 but limited to the markers GnRH, GHRH, TenM-1,-2,-3 or -4, arginin vasopressin, thyrotropin-releasing hormone, SOCS-3, urocortin, melanocortin-concentrating hormone (MCH), orexin, dopamin transporter, corticotrophin-releasing factor, gonadotrophin-releasing hormone, tryptophan hydroxylase, tyrosine hydroxylase, galanin, proopiomelanocortin, proglucagon, neurotensin, somatostatin, agouti-related protein, CART, leptin, oxytocin, corticotrophin-releasing factor receptor-1 or -2, aromatase, ghrelin, growth hormone secretagogue receptor, MCH receptor-3 or -4, NPY receptor-Y1 or -Y2, calcitonin receptor-like receptor, glucagon-like peptide receptor-1 or -2, neurotensin receptor.

Invention 2 is limited to the marker GnRH.

Invention 3 is limited to the marker GHRH.

Invention 4 is limited to the marker TenM-1,-2,-3 or -4;etc...

Invention 33 is limited to the marker neurotensin receptor.

Invention 34 : claims 18-20.

Immortalized cell line of murine hypothalamic neuronal cells that is responsive to a teneurin C-terminal-associated peptide (TCAP).